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Disease-Modification in Huntington's Disease: Moving Away from a Single-Target Approach

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## Abstract

To date, no candidate intervention has demonstrated a disease-modifying effect in Huntington's disease, despite promising results in preclinical studies. In this commentary we discuss disease-modifying therapies that have been trialled in Huntington's disease and speculate that these failures may be attributed, in part, to the assumption that a single drug selectively targeting one aspect of disease pathology will be universally effective, regardless of disease stage or "subtype". We therefore propose an alternative approach for effective disease-modification that uses 1) a combination approach rather than monotherapy, and 2) targets the disease process early on – before it is clinically manifest. Finally, we will consider whether this change in approach that we propose will be relevant in the future given the recent shift to targeting more proximal disease processes – e.g. huntingtin gene expression; a timely question given Roche's recent decision to take on the clinical development of a promising new drug candidate in Huntington's disease, IONIS-HTTRx.

## Keywords:

Huntington Disease, Clinical Trial, Oligonucleotides Antisense, Drug Therapy Combination, Pre-manifest Disease

To date, no disease-modifying therapy exists for Huntington's disease (HD), despite considerable financial investment [1]. Roche pharmaceuticals, however, believe that IONIS-HTTRx, a promising new drug candidate, bucks this trend; their milestone payment to IONIS follows completion of a phase 1/2 study of IONIS-HTTRx, an antisense oligonucleotide (ASO), in 46 patients with early-stage HD. The drug was safe and well tolerated, and resulted in dose-dependent reductions in huntingtin protein (HTT) in the cerebrospinal fluid (CSF) of treated participants [2]. Roche anticipate that results of a trial designed to assess clinical efficacy will prove definitive, accelerating FDA-approval of IONIS-HTTRx. Other global leaders in therapeutics – Spark, Voyager, and UniQure – have followed suit with programs targeting the mutant huntingtin (muHTT) mRNA, representing a shift in optimism affording by targeting the 'root cause' of the disease – HTT gene expression [3].

Despite a shift in approach – from downstream to upstream targets – drug development in HD has adopted a common mentality: searching for a single agent which selectively targets one aspect of HD pathology which would then be universally effective in this condition, regardless of when in the disease course it is administered and in what type of patient [4]. Although such a therapy has proven elusive, the selection of candidate disease-modifying therapies has been grounded in mechanistic insights into HD pathogenesis (fig. 1). Two potential explanations for such low rates of clinical success have received less consideration, and stem from adopting this mentality.

### **Reason one: combination therapy has been disregarded**

To date, it has been assumed that manipulating a single pathogenic process will be sufficient to halt disease progression. Initial attempts at disease modification focussed on counteracting one of the downstream effects of muHTT thought to be a key contributor to neuronal death: mitochondrial dysfunction [5], excitotoxicity [6], or oxidative stress [7]. These strategies failed to demonstrate efficacy in phase 3 trials, despite promising results in animal models [8-12]. Typically, candidate drugs were administered as monotherapy, and negative results triggered a shift in focus to an alternative downstream pathway, rather than consideration of a combinatorial approach targeting multiple pathways as has happened in other conditions (e.g. HIV [13]).

Support for a multi-drug strategy would require the direct comparison of the ability of monotherapy versus combination therapy to slow disease progression; an approach that has been increasingly adopted in oncology trials [14, 15]. A small number of *in vivo* studies in HD have adopted this paradigm; examining the neuroprotective effects of coenzyme Q10 administered alone or in combination with other modulators of downstream pathogenicity [16-20]. In excitotoxic rodent models of HD, co-administration of coenzyme Q10 with lamotrigine or creatine has additive effects in reducing striatal lesion volumes, which are significantly greater than those achieved by either compound alone [16, 18]. Whilst both coenzyme Q10 and creatine attenuate oxidative damage in the cerebral cortex of the 3-nitropropionic acid rat model of HD, this only reaches statistical significance when both compounds are administered together [16]. These findings have been replicated in transgenic mouse models, which more accurately recapitulate the progressive, age-dependent nature of HD than acute lesion-based models [21]. In R6/2 mice, for example, combined treatment with coenzyme Q10 and creatine, is significantly more efficacious than either compound alone in improving survival and motor performance [16]. Similar effects have been observed with different drug combinations (coenzyme Q10 with minocycline [17] or remacemide [20]) and in different transgenic models (N171-Q82 mice [19]).

In a rare attempt to examine the effect of combination therapy in patients, 347 participants with early HD were randomised to receive coenzyme Q10, remacemide, both, or neither and the treatment given for 30 months. Neither therapy, either alone or in combination, significantly slowed decline in total functional capacity, the primary efficacy measure [22]. The trial was, however, criticised for inadequate dosing and follow-up time; issues which were addressed in the subsequent 2CARE study [9]. Disappointingly, this trial evaluated only the effects of coenzyme Q10 monotherapy on disease progression, and not in combination with remacemide.

Given the pleiotropic cellular derangements apparent in HD [23], disease progression is unlikely to be affected by manipulating a single consequence of muHTT. One potential solution, as we have

hypothesised, is to simultaneously target multiple downstream pathogenic pathways, which is feasible especially when the agents being trialled have already been safely used in the clinic before for other indications. Instead, the emphasis of research has shifted upstream, for obvious reasons, to target muHTT itself, either by increasing its clearance or reducing its production [23]. Logically, modulating these critical pathogenic events should equate to modulating all of its downstream consequences, but even here targeting both the production *and* clearance of the muHTT is bound to be more efficacious than one approach alone.

Indeed, therapies aimed at reducing muHTT production are now entering the clinic and one small trial looking at increasing the clearance of muHTT through autophagy has also been undertaken [24]. Autophagy is a major degradation pathway for aggregate-prone proteins [25], and has been shown to be an important route for HTT degradation. In a proof of principle study, induction of autophagy by rapamycin, an inhibitor of mammalian target of rapamycin (mTOR), attenuated neurodegeneration in a fly model of HD and improved behavioural performance in a mouse model of HD [26]. Subsequent drug screening has identified less toxic, mTOR-independent, autophagy enhancers (such as lithium, trehalose, and rilmenidine) [25], which retain neuroprotective effects in cellular, fly, zebrafish, and mouse models of HD [25, 27-31]. Given the additive benefits of lithium and rapamycin observed in cell culture and *Drosophila* models of HD [32, 33], it has been proposed that combination therapy with mTOR-dependent and –independent compounds would maximise clearance of muHTT [32]. This is supported mechanistically: lithium both upregulates and inhibits autophagy (via its actions on IMPase and GSK-3B, respectively), but the undesirable effects of GSK-3B inhibition can be counteracted by co-treatment with rapamycin [32]. In translation of these results to the clinic, a recent open-label study found that rilmenidine was well tolerated in mild-moderate HD, but placebo-controlled trials will be needed to assess disease-modifying effect [24]. Interestingly, rilmenidine (like lithium) inhibits GSK-3B [34], suggesting that co-administration with an mTOR inhibitor, albeit with a non-toxic alternative to rapamycin, may be necessary to significantly upregulate autophagy and that this may make for a more logical next trial using this approach, possibly in combination with a HTT lowering agent. In fact, even downstream events may impact on these more proximal disease

processes, for example insults that elevate intracytosolic calcium (like excitotoxicity) inhibit autophagy [31], suggesting that maximising the benefits of autophagy may also depend on simultaneously blocking downstream pathogenic pathways.

In contrast to the strategies discussed above, attempts to ‘replace/repair’ the HD brain bypass the canonical disease mechanisms. ‘Replacement’ strategies hinge on the observation that neurodegeneration (at least in early HD) is relatively localised to the striatum; a tractable target for cell replacement therapy. Contingent on optimal tissue preparation and volume [35], human fetal striatal cell transplants produce motor and cognitive improvements in some HD patients [36, 37], but the benefit appears to be only transient [35, 37, 38]. This secondary decline emphasises that, whilst transplants may help some aspects of the condition for some time, they do not slow the underlying disease process. As HD progresses, the striatal graft becomes vulnerable to degeneration (possibly via cell non-autonomous mechanisms) [39], and the pathology in other brain regions overwhelms any benefits from the transplanted tissue. Thus if this strategy is to be adopted, a combinatorial approach will be needed: namely grafting to restore lost function, combined with mechanistic therapies to slow neurodegeneration. Furthermore, studies in rodent lesion-based models of HD have shown that striatal-specific behavioural training is required for graft-host integration and motor recovery [40-43], suggesting that graft functionality may also depend on concurrent rehabilitation [44]. Simply replacing lost neurons is insufficient; ‘learning to use the transplant’ is necessary [45] and needs to be done in the context of therapies designed to slow or reverse disease processes in structures connecting to and from the grafted cells. Indeed, recently it has been shown that wild-type human glial progenitor cell engraftment slows disease progression in R6/2 mice [46]. Thus neuronal and glial grafts may be needed to optimise any cell-based therapy for treating HD.

Reparative strategies have also sought to target neurotrophic support for medium spiny neurons (MSNs). As with circuit reconstruction, this approach is ‘pragmatic’ rather than mechanism-driven: stabilising affected functions without addressing the underlying cause of neuronal death – muHTT and its molecular consequences [45]. The reparative effects of brain derived neurotrophic factor

(BDNF), for example, have been investigated in animal models of HD [47]. Although delivery issues have been the main factor delaying the translation of trophic therapies to the clinic, concerns exist over whether BDNF will demonstrate efficacy as monotherapy. In mouse models of HD, despite adequate delivery of BDNF, TrkB receptors failed to engage post-synaptic signalling mechanisms controlling the induction of corticostriatal synapses. Plasticity could be rescued by concomitant inhibition of p75NTR signalling or its downstream target, PTEN [48]. Accordingly, the beneficial effects of BDNF may only become apparent with co-administration of compounds that restore TrkB signalling – as has been suggested for glial cell line-derived neurotrophic factor (GDNF) use in patients with Parkinson's disease [49].

Within each therapeutic strategy trialled to date, poly-therapy may therefore confer benefit over monotherapy. Integrating these approaches may also prove useful, given their complementary objectives: whilst cell repair/replacement can briefly restore function, compounds counteracting the downstream effects of muHTT may interrupt neuronal death and improve autophagy, and therapies reducing muHTT production or promoting muHTT clearance could further help affected neurons better handle muHTT [50]. Typically with poly-pharmacy, efficacy must be balanced with negative clinical consequences (increased adverse effects, drug-drug interactions and adherence issues). In the case of the oral therapies discussed (autophagy inducers and downstream modulators), however, this may be offset by the fact that lower doses of each drug are likely to be required, allowing for a larger safety window before toxicity from off-target effects [31] – as shown in combinatorial regimes for hypertension [51] and heart failure [52]. Furthermore, many of these compounds are known to be safe and well-tolerated [31, 53-57], and have a history of clinical use for other purposes.

Prioritising such new combinatorial regimes would require establishing the clinical contribution of each drug candidate. Recent FDA guidance recommends a factorial 2x2 design: a four-arm trial comparing co-administration to each drug alone and to placebo (AB v. A v. B v. placebo) [58], as employed in the CoQ/remacemide study [22]. Such designs, however, increase the required sample size – by up to eight-fold when the efficacy of each drug alone is minimal [59]. The

CoQ/remacemide study enrolled 86-87 subjects in each of the four treatment arms [22]; sufficient power to detect main effects of CoQ/remacemide against placebo, but insufficient to determine the contribution of each drug and potential interactions [59]. A proposed solution is an ‘adaptive’ factorial design: initially using the 4x4 design and terminating the monotherapy arm early if it becomes clear that single agents have minimal efficacy [58], facilitated by the use of surrogate endpoints/biomarkers (e.g. neuroimaging responses [60]) rather than rates of “disease modification”. Similar to the multi-arm multi-stage trials employed in cancer therapeutics [61], such designs address a key challenge facing factorial studies: reaching statistical power [62]. The need for combination toxicology studies, and the concomitant additional costs, may be an additional barrier to approval, and appropriate dosing for combinations will need to be established. Given these concerns, the FDA has set out criteria against which the benefits of co-development mitigate the costs [58]: (1) “the combination is intended to treat a serious disease”, (2) “there is a strong biological rationale for use of the combination (e.g. the agents inhibit distinct ... steps in disease pathogenesis)”, (3) “a nonclinical model should demonstrate that the combination has substantial activity and provides greater activity ... than the individual agents” and (4) “there is a compelling reason why the new investigational drugs cannot be developed independently (e.g. ... one or both of the agents would be expected to have very limited activity when used as monotherapy”); criteria which could easily be applied to therapeutic approaches for HD. So as in the management of common disease such as diabetes [63] and hypertension [64], a ‘staged’ approach may be suitable, with ‘intensification’ of treatment (e.g. moving from dual to triple therapy) guided by disease-related (e.g. CAG repeat length, genetic modifiers of predicted age of onset) and patient-related (e.g. biological age and comorbidities, adverse effects of prior therapy) factors.

### **Reason two: too little, too late**

Drug development to date has adopted a second assumption: that an effective therapy will slow disease progression regardless of timing of administration and/or the subtype of HD being treated. While this latter concept of disease subtypes is currently poorly understood, it is clear that patients do follow different courses and some of the genetic basis for this is being identified, both in HD [65], and



in other neurodegenerative disorders such as Parkinson's disease [66]. However disease stage has long been argued to be important in the use of disease-modifying drugs in neurology, for example in Alzheimer's disease [67] and multiple sclerosis [68, 69]. So in HD, could low clinical success rates be explained, in part, by failure to initiate therapy early? In virtually all phase 3 trials, therapies have been administered to patients with clinically manifest disease, with disappointing results [8-12]. In contrast, in successful pre-clinical studies using transgenic HD mice, treatment has typically been initiated prior to, or shortly after, the onset of overt behavioural features [70]. Creatine and coenzyme Q10, putative correctors of downstream metabolic impairments in HD, are discussed below, given the wealth of data that exists for these therapies; but similar discrepancies between timing of administration in animal models and patients can be observed with other therapies trialled, such as riluzole [71, 72], minocycline [12, 17, 73], and cysteamine [55, 74-77].

The neuroprotective effects of creatine have been extensively studied in animal models of HD and in HD patients. *In vivo*, creatine administered 2 weeks before intrastriatal administration of mitochondrial-neurotoxins protected against increases in striatal lactate and 3-nitrotyrosine, markers of oxidative stress [78]. In R6/2 and N71-82Q mice, pre-manifest creatine supplementation (at 3 and 4 weeks of age, respectively) significantly increased survival, delayed development of motor deficits and attenuated brain atrophy [79, 80]. In an attempt to model clinical trials, a follow-up study examined the effects of supplementation started at 6-, 8-, and 10-week-old R6/2 mice, analogous to the early, middle, and late stages of manifest HD [70]. Robust improvements in clinical and neuropathological phenotype were only observed with the 6-week treatment paradigm, and these were modest compared to those seen with a 3-week start time. Clinical trials of creatine in manifest HD patients (mean disease duration 8 years in a pilot clinical study [81] and 6 years in a phase 3 trial [10]) failed to show any effect on disease progression. The PRECREST trial is the only study to date assessing a candidate disease-modifying therapy in pre-manifest HD patients (pre-HD) [60].

Although primarily designed to assess the safety and tolerability of creatine, diffusion magnetic resonance imaging (MRI) was included as a secondary endpoint and potential prodromal biomarker. At the end of the 6-month placebo-controlled phase, the rate of precentral, occipital, superior frontal,

and supramarginal cortical thinning was significantly slower in the creatine, compared to the placebo, group in whom thinning progressed at a rate of 5% per year. After 12-months of open label creatine, the group crossing over from placebo demonstrated slowing of cortical atrophy, replicating the initial treatment benefit [60].

Similarly, the neuroprotective effect of coenzyme Q10 in mouse models of HD depends on early administration. Coenzyme Q10 supplementation initiated before mice exhibit behavioural dysfunction (8 weeks in the HD-N171-82Q model and 3-4 weeks in the R6/2 model), comparable to a trial in pre-HD, ameliorates motor dysfunction [19, 20, 82]. In phase 2 and 3 trials coenzyme Q10 did not significantly alter decline in functional capacity compared to placebo but, the therapy was only trialled in patients with established disease (5 and 4.7 years after symptom onset, respectively) [9, 22]. Results of the PREQUEL trial, a double-blind randomised study of coenzyme Q10 in pre-HD, are awaited [83].

Is the potential benefit of initiating therapy at pre-manifest stages substantiated at the molecular level? This would require confirmation that brain changes in pre-HD: 1) are present, thereby providing a tractable target for disease-modification; and 2) are potentially reversible, insofar as to yield greater clinical benefit than therapies administered in manifest disease (by which time striatal volume is reduced by ~50%) [84]. This depends on whether neuronal dysfunction, a potentially reversible process, predates neurodegeneration, a non-reversible process. In transgenic and knock-in mouse models of HD, there is evidence that striatal, and to a lesser extent cortical, neuronal dysfunction predates cell death and phenotypic changes. Before the onset of overt motor dysfunction, transgenic mouse models of HD exhibit disturbances in long-term depression (LTD) and short-term plasticity at cortical synapses, with subtle associated impairments in cognition and behaviour (mimicking ‘prodromal’ HD) [85-88]. Preceding this ‘prodrome’, cultured MSNs display subtle shifts in the balance between extrasynaptic versus synaptic NMDA receptor signalling, with profound consequences on downstream survival signalling [89]. Treatment of pre-manifest YAC128 mice with low-dose memantine blocks extrasynaptic (but not synaptic) NMDA receptors and delays the onset of

neuropathological and behavioural abnormalities [90, 91]. NMDA receptor blockers have been trialled in HD, with negative outcomes for riluzole [71], remacemide [22], and lamotrigine [92]. Critically, these are non-selective NMDA receptors blockers, and were administered to manifesting patients. Selective extrasynaptic NMDA receptor blockade, for example with low-dose memantine, administered within the potentially narrow therapeutic window (in pre-HD), may be more effective [93]. Encouragingly, therapy testing in animal models is increasingly capitalising on this concept of a critical therapeutic window: a recent study utilising *in vivo* two-photon calcium imaging, demonstrated that metformin given to Hdh150 knock-in mice in the ‘very far from disease onset’ stage reversed early neuronal network dysregulations observed in pre-manifest stages [94].

Whether neuronal dysfunction predates neuronal loss in patients is less well established. Given the marked differences in disease time-course, it cannot be directly inferred from animal models. In transgenic and knock-in mouse models of HD neuronal loss occurs either comparatively late (after motor dysfunction in YAC-128, knock-in CAG140 and BAC CAG97 mice), not at all (R6/1 mice), or is difficult to assess given the speed of phenotype progression (R6/2 mice) [89]. In contrast, striatal and white matter volume loss is detectable in human expansion carriers 10-15 years before manifest disease [95, 96]. Measuring motor-evoked potentials in response to transcranial magnetic stimulation (TMS) has been used to detect neuronal dysfunction in pre-HD [97, 98]. In controls, theta burst pattern TMS transiently inhibits motor cortex excitability, whereas in pre-HD individuals there is no effect [97]; reminiscent of the loss of cortical LTD observed in mouse models of HD [88]. Regional hyper- or hypo- activation on task-based functional MRI has also been used to measure neural abnormality in pre-HD [99, 100]. In an attempt to clarify whether such differences equate to neuronal dysfunction or represent compensatory responses to pre-existing structural pathology [101], close and far from disease onset pre-HD participants (~<12 and >12 years to diagnosis, respectively) underwent structural imaging and task-based functional imaging [100]. On functional imaging, far from disease onset participants demonstrated hypo-activation of caudate and thalamic regions and hyper-activation of medial prefrontal regions relative to controls. Far from disease onset participants also performed no worse than controls on the time-discrimination task, suggesting that, preceding functional

impairment, prefrontal hyperactivation compensates for reduced subcortical participation [100].

Interestingly, structural imaging revealed reduced striatal volume in close, but not far, from disease onset participants; suggesting the compensatory plasticity in far from disease onset pre-HD arises in response to striatal neuron dysfunction, before overt striatal degeneration.

Prevention trials in pre-HD face a challenge: defining clinical outcome measures. One approach may be to assess whether a therapy delays the onset of manifest disease – that is, unequivocal motor signs of HD. This would require reliable, individual-level, predictors of progression to manifest HD.

Although robust at the population level, CAG repeat length is an imprecise predictor of HD onset in individuals, as 30% of variance in age of onset is influenced by other genetic and environmental modifiers [102, 103]. Given this heterogeneity, demonstrating reduced rates of clinical onset would necessitate impracticably large sample sizes and long follow-up periods [60]. Alternatively, efficacy could be defined as a slowing of pre-manifest ‘disease’ progression. While subtle changes in clinical and neuroimaging measures occur as early as 20 years before diagnosis, robust markers of pre-manifest progression against which interventions could be evaluated remain ill-defined – but could include some of the measures mentioned above using functional imaging and neurophysiology. In recent years prospective studies (PHAROS, PREDICT-HD, and TRACK-HD) have sought to define the preclinical features of HD [84, 104, 105], by longitudinal clinical and neuroimaging assessment of cohorts of gene-expanded patients. There was broad agreement that prodromal HD could be reliably detected at the group level [101], but phenotypic markers for pre-HD remained elusive. That said, recent re-analysis of the TRACK-HD cohort suggests that assessment of ‘disease’ progression may be possible in pre-HD. The study categorized gene-expanded subjects as pre-HD A and B ( $\sim >10.8$  and  $\sim <10.8$  years from predicted onset, respectively). Changes in 3-T MRI and clinical assessments were measured over 36 months. Although, the pre-HD A group were virtually indistinguishable from controls, the timed tapping test and grey-matter volume emerged as reliable predictors of phenotypic progression [96]. Although these markers lack the sensitivity and specificity necessary for predicting disease onset in individuals, at the population level they offer a potentially robust method for evaluating pre-HD ‘disease’ progression [101].

Another hurdle for prevention trials is that most individuals with a genetic risk of HD do not come forward for genetic testing (>80% in the UK) [106], for reasons that are not clear but may include emotional distress and risk of genetic discrimination [60, 107]. As such, the pool of potential participants (confirmed genetic carriers) is low and genetic testing as a condition for inclusion could be considered coercive. In a novel attempt to address these challenges, the PRECREST trial recruited both 50% at-risk individuals and confirmed carriers [60]. Double-blind genotyping was used to establish the diagnosis in the at-risk group, and those testing negative were incorporated into the healthy control group. This strategy increased the pool of pre-HD individuals available and prevented coercion of testing.

### **IONIS-HTTRx: a single-target cure?**

In contrast to therapies trialled to date, IONIS-HTTRx represents a novel approach (fig. 1). Could this therapy therefore represent a single-agent ‘cure’, that proves efficacious regardless of timing of administration? ‘Gene silencing’ strategies aim to reduce production of muHTT, by inhibiting transcription of the HTT gene or translation of its cognate mRNA. RNA-targeting strategies have received more attention, given that mRNA is more readily accessible than DNA and is unprotected by repair machinery [23]. IONIS-HTTRx, an ASO, is the first RNA-targeting strategy to transition from *in vivo* testing to clinical trials in HD. IONIS-HTTRx are synthetic, single-stranded oligonucleotides which bind by complementary base pairing to HTT mRNA, triggering its endonuclease-mediated degradation [108]. In a mouse model of HD, intrathecal infusions of ASOs targeting HTT led to dose-dependent reductions in HTT mRNA, reversal of the disease phenotype and improved survival; providing the therapeutic rationale for clinical trials [109].

Results of the phase 1/2 trial were promising, but heralding IONIS-HTTRx as a ‘cure’ may be premature. Indeed, although the degree of muHTT lowering was associated with positive signals on several UHDRS outcomes on the last day of dosing, these analyses were post-hoc, and there were no significant improvements in any active dose group versus placebo in clinical measures defined *a*

*priori* [110], although the numbers of patients treated were small and not powered to see such changes. Indeed, the trial was designed to assess safety and tolerability: larger trials, with adequate follow-up will be needed to establish whether IONIS-HTTRx slows disease progression [2]. As with other therapies trialled to date, IONIS-HTTRx was administered as monotherapy, and in manifest (albeit early stage) disease. Arguably, in this instance, this is justified: the therapy targets the *most* proximal pathogenic event – the root cause of the disease – so may achieve clinical benefit in isolation, regardless of timing of administration; as Roche no doubt expect of IONIS-HTTRx. A combinatorial approach, however, may still be warranted. Gene silencing therapies face a common challenge: selectively lowering muHTT whilst minimising suppression of wild-type HTT (wtHTT). Although muHTT is the primary mediator of disease, loss of wtHTT may contribute to pathogenesis [111]. In a zebrafish model of HD, ASO-mediated knockdown of wtHTT halved BDNF levels [112], a critical survival factor for MSNs and modulator of corticostriatal synaptic function [111]. A number of allele-specific silencing approaches are in development, such as selective targeting of CAG expansions [113-115], but these have resulted in detrimental downregulation of other CAG-containing transcripts [113, 115]. An alternative approach is to target small nucleotide polymorphisms variants enriched on the mutant allele [116-118], but this is unlikely to be scalable, reproducible, or cost-effective [119]. A combinatorial ‘suppression and repression’ strategy, involving co-administration of short interfering RNA (for non-allele-specific gene suppression) with gene therapy (to supplement wtHTT), may overcome these issues [119]. This approach has shown promise in mouse models of dominant retinitis pigmentosa [120] and alpha-1 antitrypsin deficiency [121], and may represent a viable strategy for IONIS-HTTRx which is expected to reduce expression of both muHTT and wtHTT [23].

It is also uncertain whether gene silencing treatment initiated at manifest disease stages can actually reverse neuropathology and HD features. It is useful to draw on evidence from RNAi compounds, given that preclinical studies have utilised both pre-manifest and manifest mammalian models of HD. RNAi compounds and ASOs both trigger the degradation of HTT mRNA: RNAi target mature, spliced cytosolic mRNA, whereas ASOs target pre-mRNA [23]. Preclinical studies, in rodents and

non-human primates, have shown that RNAi reduces HTT mRNA levels, decreases the number of HTT inclusions, and delays the onset of behavioural deficits [119]. In many of these initial *in vivo* studies, however, RNAi treatment was initiated in pre-manifest animals, so limited conclusions about phenotypic reversal could be drawn [122-127]. In subsequent studies in rodents with manifest disease, RNAi only partially reversed the number of HTT inclusions and striatal function relative to pre-manifest administration, and behavioural outcomes either did not improve [128], or were not reported [119, 129, 130]. Less *in vivo* data exists for ASOs, and the timing of the intervention is frequently not specified [116, 118]. One study, however, found that ASOs administered to YAC128/BACHD mice with manifest disease improved both neuropathology and motor function up to 3 months post-treatment [109]. Interestingly, in the YAC128 model late initiation of ASO therapy (at 6 months) did not significantly improve motor performance compared to saline-treated YAC128 mice, despite a 44% reduction in muHTT. In contrast, administration at a younger age (3 months), near the onset of overt motor dysfunction, restored rotarod performance to non-transgenic control levels after 2 months [109], suggesting that the efficacy of ASOs may depend on early administration, before the downstream consequences of muHTT are fully established. In a follow-up study in non-human primates, intrathecal delivery of ASOs for 3 weeks led to a sustained (1 month) reduction in the levels of muHTT mRNA in the frontal cortex, occipital cortex, and the spinal cord, but behavioural outcomes were not reported and age at administration was not specified [109]. Encouragingly, it has been demonstrated in mice that continuous production of muHTT may be necessary to maintain disease progression, and that muHTT can be cleared well after manifest neuronal dysfunction [131, 132].

Evidence from other neurodegenerative proteinopathies suggests that targeting the pathogenic protein, while producing phenotypic reversal in manifest animal models, may not necessarily modify disease course in manifest patients. One approach to reduce amyloid beta (A $\beta$ ) in Alzheimer's disease (AD), for example, involves using solanezumab, a humanised monoclonal antibody which binds to and promotes the clearance and/or sequestration of soluble amyloid [133]. In phase 3 trials [133, 134], solanezumab did not improve cognition or function in mild-to-moderate [133] and mild [134] AD,

despite decreases in the CSF levels of unbound A $\beta$ , consistent with target engagement of soluble brain amyloid. In contrast, passive immunisation with anti-A $\beta$  antibody well after the onset of behavioural deficits reverses memory impairments in old (24 months of age) PDAPP transgenic AD mice [135]. It has been suggested that in manifest AD patients, neurodegeneration has become self-propagating and not susceptible to intervention with anti-amyloid drugs. Clinical trials testing anti-amyloid therapies in individuals at risk of AD based on elevated intracerebral amyloid A $\beta$  [136] and/or genotype e.g. PSEN1 E280 [137] and APOE4 carriers [138], may address this question. Having said that, there is increasing evidence that AD is characterised by a two-stage process in which amyloid initiates a cascade of downstream neurotoxic effects, which become increasingly ‘amyloid-independent’; potentially explaining the lack of efficacy of targeting A $\beta$  in established disease [139]. To date, we do not have evidence of a similar biphasic mechanistic hypothesis in HD.

Notwithstanding these issues, an additional obstacle to the progress of ASOs to the clinic has been the lack of a safe, efficacious, and non-toxic delivery system. Systemic administration results in poor central nervous system (CNS) distribution, owing to the impermeability of the blood-brain barrier (BBB) to oligonucleotides [108]. Intraparenchymal administration mediates robust localised delivery but is invasive [140-142]. Intrathecal administration, is safe and circumvents restrictions imposed by the BBB, but does not guarantee targeted delivery to all affected neurons and creates logistical issues in clinical translation. Indeed, in the IONIS-HTTRx trial, ASOs administered intrathecally modulated CSF HTT protein concentrations, but adequate brain exposure was not confirmed [2]. Arguably, results from trials of the ASO nusinersin in spinal muscular atrophy (SMA) help alleviate some of these concerns. In a phase 3 trial, nusinersin administered intrathecally to infants with SMA increased overall survival and attainment of motor milestones compared to sham procedure [143]. Analysis of autopsy tissue showed that nusinersin distributed broadly throughout the spinal cord and brain parenchyma, at therapeutic concentrations [144]. However, intrathecal pharmacokinetics and CNS anatomy vary with age [145], limiting the generalisability of these conclusions to adult populations. Drug penetration from the CSF into the brain parenchyma may also be age-dependent:



in rats, intrathecal delivery of a green fluorescent protein (GFP) tagged adeno-associated viral vector in neonates results in stronger GFP expression, and more selective neuronal tropism, than in adults [146]. Proposed explanations include: (i) higher water content in the immature brain, increasing the CSF-parenchyma concentration gradient, and (ii) increased activity of matrix metalloproteinases during development, facilitating the cleavage of extracellular proteins, and subsequent migration of neurons to the brain-CSF interface [146]. If both points hold true in humans – as evidence suggests [147, 148] – ASO exposure following intrathecal delivery may not be directly comparable in infant SMA and adult HD patients.

## **Conclusion**

No candidate therapy has demonstrated robust disease-modifying effects in HD, despite promising results in preclinical studies. The ‘single target’ mentality adopted in drug development may have hindered the consideration of two principles. Firstly, that combinations of compounds targeting multiple pathways may confer advantage over monotherapy, and this includes therapies looking to affect muHTT production. And secondly, that disease-modification may depend on initiating therapy early, before neurodegeneration has reached a point of irreversibility. There are other reasons for these failures, namely difficulties in identifying and engaging the critical pathogenic steps, determining optimal dosing regimens and the limitations of animal models in recapitulating the disease process in humans (reviewed elsewhere e.g. [21]), but the principles suggested here offer a pragmatic strategy moving forward, and add urgency to the search for markers of disease progression in pre-HD.

The idea that these two principles are key to clinical efficacy is not new. Goldie and Coldman’s seminal results in the 1980s demonstrated that this strategy reduces the emergence of resistance in cancer cells, maximising the probability of remission [149-152]. A similar approach has been successfully applied to the management of several diseases, ranging from HIV [153] and TB [154] to rheumatoid arthritis [155]. It is curious that HD, a disease genotypically present at birth, takes decades to manifest clinically. During this pre-manifest period, compensatory clearance of muHTT is

presumably adequate until, for unknown reasons, the pathological process becomes overwhelming, and protein aggregation and/or the downstream effects of muHTT cause neuronal death. Analogous to Goldie and Coldman's model, this represents a gradual shift from cellular 'sensitivity' to 'resistance'. Multiple agents, administered early in the disease process when neuronal dysfunction is potentially reversible, could incrementally delay the onset of neuronal 'resistance', prolonging the period when expansion-carriers are pre-manifest. By adopting a different strategy, disease-modification in HD may be attainable, but this is unlikely to come as a 'single agent' cure – as many companies are working towards. Ongoing trials will determine whether IONIS-HTTRx refutes this hypothesis.

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#### Conflict of Interest:

There are no conflicts of interest to disclose.

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